Effect of an Acid Antimicrobial on Turkey Breast Meat *Salmonella* and Total Aerobes

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**Abstract:** Acid solutions have been used as antimicrobials on meat and poultry. FreshFx is a commercially-available surface treatment for the reduction of pathogens and extension of shelf life of raw poultry. Two replicate trials were conducted to determine the effect of FreshFx on numbers of total aerobes and inoculated *Salmonella* on raw turkey breast meat during 14 d of refrigerated storage. In each trial, three turkey breast meat cutlets, obtained at retail, were inoculated with nalidixic acid-resistant *Salmonella* and cut in half. One half was dipped in FreshFx for 30 s and the other half dipped in water for 30 s as a control, then all cutlet halves were refrigerated at 4°C and sampled at 0, 1, 2, 7, 10 and 14 d for total aerobic bacteria and *Salmonella*. No reduction (P>0.05) in numbers of total aerobes or *Salmonella* was observed due to FreshFx. Mean log cfu for total aerobes was 7.1 on d 0 and 7.8 on d 14. Mean pooled *Salmonella* log cfu were 6.9 on d 0 and 5.1 on d 14. Total aerobic counts were artificially high on d 0 to 10 due to the large numbers of *Salmonella* initially inoculated onto the samples. The persistence of *Salmonella* on samples during refrigerated storage reinforces the concept of reducing or eliminating pathogens prior to packaging and cold storage. In this experiment FreshFx did not improve shelf life as measured by reduction of total aerobes during refrigerated storage, nor reduce numbers of inoculated *Salmonella* on raw turkey breast meat.

**Key words:** Turkey meat, *Salmonella*, shelf life, acid antimicrobial

**INTRODUCTION**  
The growth of the turkey industry has been facilitated by the diversification of product form, from the traditional whole carcass to many types of raw and further processed items available at retail. The importance of food safety, in regard to numbers of both spoilage and pathogenic bacteria on product, has been studied as the industry expanded. Several experiments have been conducted on the effects of basic processing steps, from slaughter to chilling, on numbers or prevalence of *Salmonella* on turkey carcasses (Walker and Ayres, 1959; Campbell et al., 1984; Clouser et al., 1995). The effect of extended chilling and cold storage at various temperatures and times on psychrophilic organisms and pseudomonads has been reported for turkey carcasses (Barnes and Shrimpton, 1968). A review provided evidence that additional handling and processing past the whole carcass form resulted in higher *Salmonella* prevalence (Kraft, 1971). Processed turkey meat, including parts (e.g., drumsticks), deboned thigh meat and mechanically deboned meat were inoculated with *Salmonella* and held at 10°C or 1°C (Denton and Gardner, 1986). *Salmonella* survived or increased in numbers by 13 d of storage at either temperature. *Salmonella* contamination of commercial turkey meat is likely, as previous reports show that commercial turkeys at slaughter had a prevalence of 33.3%, while post-chill swabs from baseline data collected during 1997-1998 were 19.6% positive (Rostagno et al., 2006; Eblen et al., 2005).

Methods or applications to reduce pathogens and improve shelf life of turkey products are needed. Some recent efforts have included processing, packaging or further processing changes. Steam or boiling water improved microbiology of turkey skin but is not commercially feasible (Avens and Morton, 1999). Modified atmosphere packaging increased the shelf life of turkey cutlets but not ground turkey meat (Fraqueza and Barreto, 2009; Sauzier et al., 2000). Marination of turkey meat with organic acids improved the shelf life and decreased *Listeria* on subsequently produced deli loaves (Carroll et al., 2007; Lloyd et al., 2009). Other reports utilizing chicken meat have shown an improvement in shelf life and reduction of *Salmonella* from applications of lactic acid (Gulmez et al., 2006; Zhongping et al., 1998; Xiong et al., 1998). FreshFx is a commercially available acid solution approved for use in poultry processing as an antimicrobial. The objective of this experiment was to determine if FreshFx applied to raw turkey breast meat would initially reduce numbers of inoculated *Salmonella*, or control total aerobes during refrigerated storage.

**MATERIALS AND METHODS**  
In each of two replicate trials, three packages of raw turkey breast meat cutlets were purchased from a local grocery store. One cutlet was removed from each package and inoculated with 1 mL of a mixture of nalidixic acid-resistant *Salmonella* serotypes; 0.5 mL
which was massaged into one side of the cutlet for 10 s with a sterile spreader, held for approximately 7.5 minutes, then the other side of the cutlet was similarly treated (0.5 mL massaged into fillet for 10 s and held for 7.5 min) to allow bacterial attachment to the cutlet. Each cutlet was then cut into approximately equal halves, with one half designated as Control and the other half designated as Treated, resulting in three Control and three Treated paired halves per trial. The Salmonella inoculant mixture was prepared by placing each of four cultures (S. Enteritidis, S. Heidelberg, S. Montevideo and S. Typhimurium, all resistant to nalidixic acid), into 9 mL of Brain Heart Infusion broth (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37°C for 18 h. The inoculant mixture was prepared by adding two mL from each broth culture to a sterile tube and mixing. The inoculant was serially diluted and plated onto Brilliant Green Agar (BGA) with sulfapyridine (Neogen Corp., Lansing, MI) with 200 ppm nalidixic acid (Fisher Scientific, Fair Lawn, NJ) and incubated at 37°C for 24 h to determine the approximate number of Salmonella bacteria added in each trial. The numbers of Salmonella inoculated onto the samples in Trial 1 were 9.3 log10 cfu and 8.9 log10 cfu in Trial 2. The inoculated cutlet half designated as Control was dipped for 30 s into tap water while the other paired half, designated as Treated, was dipped for 30 s into an acidic antimicrobial solution, a 0.4% solution of FreshFx (Sterix, Shreveport, LA). The solution was prepared by adding 4 mL of FreshFx to 1 liter of tap water (from the same source as that used for Control); the measured pH of the 0.4% solution was 1.8. After dipping, each cutlet half was allowed to drip for 5 s and then placed into a plastic bag. A rinse solution, 50 mL of 1% buffered peptone water (BPW, Oxoid Ltd., Basingstoke, Hampshire, UK) was added to each bag which was then manually shaken for 1 min. Cutlet halves were transferred into new plastic bags after rinsing. The same three Control and three Treated cutlet halves that were sampled on day 0 were also sampled (by rinsing and re-bagging as previously described) on d 1, 2, 7, 10 and 14 in each trial and all samples were held in an incubator maintained at 4°C. After each rinse sampling, one mL from each rinseate was serially diluted in BPW and plated onto duplicate plates of BGA with sulfapyridine with 200 ppm nalidixic acid and incubated at 37°C for 24 h. Serial dilutions were also plated on duplicate Standard Methods agar (Thermo Fisher Scientific, Lenexa, KS) and incubated at 35°C for 48 hours. After incubation, colonies indicative of Salmonella were counted, as were the total aerobes present on Standard Methods agar. Bacterial counts were transformed to log10 cfu per mL of rinseate. Data were analyzed using the SAS ANOVA procedure to determine main effects of Trial and Treatment; as no significant interactions (P<0.05) were detected the means were pooled across Trial (SAS, 2004). SAS paired t test procedure was used to determine significant differences in bacterial numbers between Control and Treatment samples.

RESULTS AND DISCUSSION

There were no differences (P>0.05) in numbers of total aerobic bacteria between Control and Treated turkey meat samples (Table 1). The high number of total aerobes as seen on Days 0 through 7 may have been an artifact of the high inoculation level of Salmonella which grew on the Standard Methods media. The psychrophilic and psychrotrophic organisms more representative of spoilage bacteria were evident by Days 10 and 14, as the total aerobic count appeared to increase, while numbers of Salmonella did not, as shown in Fig. 1.

![Fig. 1: Numbers (log cfu) of total aerobes and Salmonella recovered from turkey breast meat cutlets inoculated with Salmonella after 0 to 14 days of storage at 4°C (n = 12)](image)

There was no difference (P>0.05) in Salmonella counts between Control and Treated samples on any d of storage as shown in Table 1. Numbers of Salmonella decreased during storage but were still at log 5.1 on Day 14. The competition from increasing numbers of total aerobes may have contributed to the decrease in Salmonella numbers. Although numbers of Salmonella decreased by one log after one d of storage, an additional 13 d of refrigeration resulted in an additional reduction of only one log. This slow reduction shows the importance of reducing or eliminating Salmonella on turkey meat during initial processing, as it persists on the product for a number of days during refrigeration. The growth of total aerobes and Salmonella, with combined Control and Treated samples over 14 d of refrigeration, are shown in Fig. 1. During sampling the bags were evaluated for odor and some off odor was detected at Day 10 when log counts were 6.6. At Day 14 an obvious spoilage odor was detected, along with
Table 1: Total aerobic bacteria and *Salmonella* counts (log$_{10}$ cfu/mL rinsate) of turkey breast meat cutlets dipped in water (Control) or a 0.4% vol/vol of FreshFx acid antimicrobial solution (Treated) and held at 4°C from 0 to 14 days ($n=6$)

<table>
<thead>
<tr>
<th>Day of refrigerated storage (log$_{10}$ cfu/mL)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>7</th>
<th>10</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total aerobes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.9</td>
<td>5.9</td>
<td>5.7</td>
<td>5.6</td>
<td>6.7</td>
<td>7.8</td>
</tr>
<tr>
<td>Treated</td>
<td>7.2</td>
<td>6.1</td>
<td>5.7</td>
<td>6.0</td>
<td>6.5</td>
<td>7.8</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.09</td>
<td>0.06</td>
<td>0.03</td>
<td>0.10</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Probability</td>
<td>0.12</td>
<td>0.09</td>
<td>0.04</td>
<td>0.11</td>
<td>0.52</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.7</td>
<td>5.6</td>
<td>5.5</td>
<td>5.3</td>
<td>5.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Treated</td>
<td>7.0</td>
<td>5.9</td>
<td>5.7</td>
<td>5.5</td>
<td>5.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.10</td>
<td>0.04</td>
<td>0.05</td>
<td>0.08</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Probability</td>
<td>0.14</td>
<td>0.10</td>
<td>0.20</td>
<td>0.22</td>
<td>0.32</td>
<td>0.55</td>
</tr>
</tbody>
</table>

obvious slime when log counts reached 7.8. One previous report cited spoilage occurring between log 6.0 and 7.0, while another study reported spoilage occurring at log 8.0 (Thomson et al., 1984; Mounsey, 1966). Numbers of total aerobes in this study indicate spoilage may have occurred between log 6.6 and 7.8.

Research reports published more than 100 years ago showed that cold storage affects growth of bacteria and extends shelf life (Wiley et al., 1908). In this study, although excessive numbers of mesophiles were inoculated onto the cutlets, refrigeration maintained apparent product integrity for at least seven days.

FreshFx ingredients include water, citric acid, phosphoric acid and hydrochloric acid. Previous reports have shown that organic acids applied as a marinade ingredient on turkey breast meat extended the shelf life of cooked deli loaves produced from that meat and kept in refrigerated storage. However, the exposure time of the meat to the acid was 1 h in the first report (Carroll et al., 2007) and 2 h in the second report (Lloyd et al., 2009) and obviously the post marinade treatment of the meat for both of those reports was much different than procedures outlined in this report. The repeated sampling of the same cutlets over several days using 1.0% BPW could have ameliorated the effect of FreshFx due to the buffering capacity of the rinse. However, the buffering capacity of the large surface area of the meat cutlet and leakage of sarcoplasmic proteins and fluid would likely exceed that of the BPW. A prior report showed that post rigor broiler breast had higher buffering capacity than other cuts of beef, pork, or leg or thigh broiler muscles (Puolanne and Kivikari, 2000). Also, the buffering capacity of BPW from Oxoed used in the present study has been shown to be lower than another brand of BPW (Baylis et al., 2000). Potentially these larger portions of breast meat cutlets may not show a reduction in *Salmonella* numbers, since the application time was only 30 s and the buffering capacity of the meat itself may quickly negate the pH decrease typically provided by the FreshFx dip, more so than the rinse of BPW. For example, a single turkey breast cutlet was immersed in 50mL of prepared FreshFx solution for 30 s and the pH of the solution increased from 1.9 to 2.8.

FreshFx as applied in this experiment had no effect on reducing *Salmonella* contamination or extending shelf life. Although experimental conditions (high numbers of bacteria inoculated onto meat samples with ample attachment time) represented a considerable challenge, some reduction of *Salmonella* was expected. The high inoculation level of *Salmonella* and its subsequent survival, artificially inflated the total aerobic bacteria count in the first few days of the experiment. Further research is needed to determine effective methods of reducing or eliminating *Salmonella* on raw turkey meat.

REFERENCES


